EFFECTS OF *HELICOBACTER PYLORI* ON THE PATHOGENESIS OF CHRONIC URTICARIA

PhD Thesis

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Contents

Abbreviations	3
List of full papers serving as background for the thesis	4
Summary	5
Introduction	7
Chapter 1	14
Effects of *Helicobacter pylori* eradication on chronic urticaria	
1.1. Patients and methods	14
1.2. Results	15
1.3. Discussion	16
Chapter 2	21
Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria (effects of *Helicobacter pylori* on pathogenesis of autoimmune urticaria)	
2.1. Patients and methods	21
2.2. Results	22
2.3. Discussion	23
Chapter 3	27
High prevalence of IgG and IgA antibodies to 19 kD *Helicobacter pylori*-associated lipoprotein in chronic urticaria	
3.1. Patients and methods	27
3.2. Results	28
3.3. Discussion	29
References	35
Publications supporting the thesis	44
Publications indirectly connected with thesis	45
Acknowledgements	48
Annex (enclosed full papers)	49

Abbreviations

CagA	cytotoxicity associated gene A
CU	chronic urticaria
FcεRIα	α-chain of the high-affinity IgE receptor
hcpA	*H. pylori* cystein-rich 28 kD protein
HLA	histocompatibility leucocyte antigen
H. pylori	*Helicobacter pylori*
hsp	heat shock protein
Ig	immunoglobulin
lpp20	lipoprotein 20
OMP	outer membrane protein
T4	thyroxine
TG	antithyroglobulin antibody
TSH	thyroid-stimulating hormone
TPO	antithyroid peroxidase
VacA	vacuolating cytotoxin A

List of full papers serving as background for the thesis

I. **Bakos** N, Szántó H: A *Helicobacter pylori* patogenetikai szerepe krónikus urtikáriában. (Pathogenetic role of *Helicobacter pylori* in chronic urticaria.) *Bőrgyógy. és Venerol. Szemle* 1998; 74: 9-13.

II. a. **Bakos** N, Hillander M: Vizsgálatok autoimmun urtikáriában. (Investigations on autoimmune urticaria) *Bõrgyóg.y és Venerol. Szemle* 2002; 78: 157-160.

b. **Bakos** N, Hillander M: Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria. *Int. J. Dermatol.* (in press)

III. **Bakos** N, Fekete B., Prohászka Z, Füst G, Kalabay L: High prevalence of IgG and IgA antibodies to 19 kd *Helicobacter pylori* associated lipoprotein in chronic urticaria. *Allergy* (in press)

Summary

The role of *Helicobacter pylori* in the pathogenesis of chronic urticaria (CU) has been questioned until recently. It is tempting to speculate that it triggers urticaria by means of an immunoglobulin E (IgE)-mediated pathway. *H. pylori*-specific IgE and consecutive mast cell and eosinophil activation take part in a local inflammatory process due to infection and in *H. pylori*-associated gastritis and ulcer. I have demonstrated an elevated total serum IgE level as compared with that in seronegative CU patients (I). Nevertheless, *H. pylori*-specific IgE is not common in CU, and there might not be a link between *H. pylori* and CU.

My findings have strengthened a beneficial effect of eradication therapy on the course of CU (I). After a follow-up of 12 weeks, there was a significant difference in the symptoms of previously seropositive, but eradicated and seronegative chronic idiopathic patients. The resolution of chronic inflammation in *H. pylori* gastritis is slow, and the healing takes several months. This explains the observation that the healing or improvement of CU occurred within 3-12 weeks after successful eradication.

Thes data support the hypothesis that *H. pylori* could play an indirect role as a triggering factor in the pathogenesis of CU.

H. pylori has several immunoreactive proteins, which take part in humoral and cellular immunoreactivity. Antigastric antibodies play a role in the pathogenesis of gastric mucosal atrophy and gastritis. Type A gastritis (corpus-restricted autoimmune gastritis) is often associated with pernicious anaemia, an autoimmune disease. Immunoreactivity against some *H. pylori* proteins, mainly structural proteins and products, leads to cross-reactivity between essential human and *H. pylori* proteins, which gives rise to extradigestive autoimmune diseases.

Previous studies have demonstrated a relation between the results of autologous serum tests and *H. pylori*, which suggests its role in autoimmune urticaria. I have found a connection between autoimmune urticaria and the most frequent organ-specific autoimmune disorder, autoimmune thyroiditis. The association was modified by the presence of *H. pylori* (II).

A further possible link might be a *H. pylori*-specific 20 kD lipoprotein. Lipoprotein may act as an autoimmune target protein in several autoimmune diseases (systemic lupus

erythematosus and rheumatoid arthitis). I have demonstrated a significant difference in immunoreactivity against lipoprotein20 between CU and non-CU patients (III).

In conclusion, my findings support the hypothesis that *H. pylori* infection may be involved in the pathogenesis of CU. However, it may be not the primary cause of CU, but rather a triggering factor involved in several different pathways. Accordingly, the eradication of *H. pylori* infection is required in seropositive patients because this may have a beneficial role in the course of CU.

Introduction

Chronic urticaria (CU) is one of the most frequent skin disorders. My survey of 18,000 normal adults in Szolnok County permitted an estimate that approximately 0.28% of the Hungarian population have CU (*Bakos 2002*). A population study in Sweden indicated that 0.11% of the men and 0.14% of the women suffered from CU (*Hellgren 1972*).

CU is not a single disease. There are a number of subsets that exhibit both clinical similarities and differences. Physical urticaria and urticaria vasculitis manifest striking differences as compared to "regular" urticaria in the clinical picture, histopathology and aetiology. Accordingly it is necessary to distinguish these subsets from CU.

CU is a polyaetiological disease. A number of factors have been identified that appear to be important in the pathogenesis of CU, including immunologic, pseudo-allergic reactions, some internal diseases and infections. Most patients believe that they have food allergy. This can be confirmed as a cause of CU in only 1% of all cases. Food additives can be demonstrated to be causative in no more than 5% (*Greaves 2000*). Chronic infections and infestations, including "focal" bacterial infections, candidiasis, parasites and protozoa such as *Enterobius vermicularis*, *Giardia lamblia, Shistosoma*, and *hepatitis B* and *C viruses* are relative often (15%) attributed as possible initiating factors of CU (*Henz et al. 1998, Trachsel et al 1999*).

In 1986, the presence of a serum factor was reported that caused whealing in response to autologous intradermal serum in some patients with CU (*Grattan et al. 1986*). In 1993, an immunoglobulin G (IgG) autoantibody directed against the α-chain of the high-affinity IgE receptor (FcεRIα) was identified (*Hide et al 1993*). This causes whealing upon autologous injection into the patient's skin and releases histamine from human mast cells and basophils (*Sabroe et al. 1998*). Subsequent studies have indicated that 25-45% of patients with CU have anti- FcεRIα and 5% anti-IgE antibodies (*Fiebiger et al. 1995, Niimi et al. 1996*). The anti-FcεRIα antibodies belong predominantly to the complement-fixing subtypes IgG1 and IgG3 (*Fiebiger et al. 1998*). IgG anti-FcεRIα antibodies cause direct cross-linking of adjacent receptors, thus triggering mast cell or basophil activation (*Greaves 2000*). This process requires complement activation, and C5a anaphylatoxin in particular (*Fiebiger et al. 1998, Ferrer et al. 1999*). Only CU patients have been shown to manifest

functional histamine-releasing anti-FcεRIα autoantibodies. Immunoreactive non-histamine-releasing FcεRIα autoantibodies have been found in autoimmune connective tissue diseases, autoimmune bullous diseases and in physical urticaria, and in other skin disorders such as atopic eczema (*Fiebiger et al. 1998, Greaves 2000*), but they have beenfound to be mainly of the IgG2 or IgG4 subtypes (*Fiebiger et al. 1998*).

The skin-restricted distribution of the symptoms of CU could be explained by the fact that this activation occurs only in dermal mast cells, and not in other organs and tissues. The reason is not exactly clear. *In vitro*, lung and other non-cutaneous mast cells release histamine in response to anti-FcεRIα autoantibodies (*Niimi et al. 1996*). Lung mast cells are unresponsive to activated complement. Mast cells of the skin, but not of the lung or intestinal mucosa, express C5aR (*Füreder et al. 1995*).

Patients with autoantibodies (anti-FcεRIα or anti-IgE) compose a subset of CU, autoimmune urticaria, but they have no distinctive clinical features. They exhibit exceptionally low peripheral basophil numbers and reduced basophil histamine releasibility (*Sabroe et al. 1999a*). Histologic examination reveals pronounced eosinophil degranulation (*Sabroe et al. 1999b.*).

The diagnosis of autoimmune urticaria is based on autologous serum skin tests. A positive test is suggestive, but not diagnostic; confirmation is needed by the *in vitro* testing of autoantibodies by ELISA, Western blotting or basophil histamine release (*Greaves 2000*). A dilution of 1:10 and 1:100 of autologous sera for screening has been suggested to separate aspecific positivity (*Husz 2002*).

The clinical course of autoimmune urticaria does not differ markedly from that of non-autoimmune urticaria, but the patients have more severe urticaria, which tends to be prolonged and less responsive to antihistamine therapy (*Greaves 2002*).

There is also evidence of an association of autoimmune diseases in some patients with CU. Our results revealed specific and non-specific markers of polysystemic autoimmune diseases in CU. The most common finding was a non-specific marker of rheumatoid factor (9.5%) (*Bakos et al. 1995*). Similar results have been reported (*Ryhal et al. 2001*).

The prevalence of thyroid autoimmunity in CU is as high as 14-20% (*Leznoff et al. 1983, Ryhal et al. 2001*) and there is also an increased prevalence of CU in autoimmune

thyroid disease (*Lanigan et al. 1987*). The association of non-endocrine organ-specific autoimmune diseases with autoimmune thyroiditis is well known as autoimmune polyglandular syndrome.

A positive autologous serum skin test was previously described in patients with urticaria associated with thyroid autoimmunity (*Gaig et al. 2000*). This suggests a correlation of chronic autoimmune urticaria and autoimmune thyroiditis in the same way as other organ-specific autoimmune diseases. The pathomechanism of the connection between thyroidal autoimmunity and CU, and especially autoimmune urticaria, is not clear. One possible explanation is the influence of a non-specific inflammatory mechanism (*Leznoff and Sussman 1989*) or the thyroidal effects in many cytokines (*Turktas et al. 1997*). Leznoff and Sussman suggested that patients with CU continuously produce excess quantities of cytokines and this is related to the development of autoimmune thyroiditis. Against this hypothesis is the fact that autoimmune thyroiditis may precede CU by several years, whereas other patients have a remission in CU with continuation of the thyroid disease. Further speculation is a cross-linking of the IgE receptors of mastocytes induced by antithyroid antibodies (*Delevaux et al. 2001*).

Recent investigations have demonstrated a strong association of autoimmune urticaria with histocompatibility leucocyte antigen (HLA) DRB1*04 (DR4) and its associated allele, DQB1*0302 (DQ8) (*O'Donnell et al. 1999*). Several studies have been preformed regarding the genetic suspectibility to autoimmune thyroid disease and have determined a strong association of Graves' disease with HLA haplotype DRB1*03 (DR3) in a Caucasian population (*Hunt et al. 2001*). On the other hand, patients with multiple autoimmune diseases, i.e. Graves'disease, type 1 diabetes mellitus and Hashimoto thyroiditis, shared the HLA haplotypic variant of DR4-DQ A1*0301 and DQB1*0302 (*Einarsdottir et al. 2003*). I hypothesize that genetic susceptibility to autoimmunity might predispose to associated autoimmune disorders such as autoimmune urticaria and autoimmune thyroiditis. The susceptible HLA loci could be responsible for the coexistence of the two diseases.

In the past decade, there have been many conflicting studies concerning CU and its association with *Helicobacter pylori* infection.

H. pylori is one of the most frequent human bacteria; more than half the world's population harbour *H. pylori*. It is generally accepted that *H. pylori* infection plays an aetiologic role in the development of chronic active gastritis, peptic ulcer disease, gastric adenocarcinoma and low-grade gastric mucosa-associated lymphoid tissue lymphoma. Recently, a potential role of *H. pylori* infection in several extraintestinal pathologies has been suggested (*Realdi et al. 1999*).

H. pylori affects a high proportion of patients with CU, but the seroprevalence does not differ from that for the normal population. Bacterium eradication is associated with a remission of the CU symptoms, suggesting its possible role in the pathogenesis of CU (*Rebora et al. 1995, Di Campli et al. 1998, Wedi et al. 1998, Wustlich et al. 1999*), but contradictory results have also been published (*Becker et al. 1998, Valsecchi et al. 1998, Schnyder et al. 1999*).

Previously, the main attention focussed on the humoral immunoreactivity against *H. pylori*. Both local and circulating antibodies can be demonstrated in infected patients. Serum anti-*H. pylori* IgG antibody titres are significantly correlated with the severity of inflammation. The decreased IgG, IgM and IgA antibody titres after eradication are of diagnostic value. The screening of serum IgG and IgA antibodies is widely used in the diagnosis of the infection. Hungarian authors first described an increased level of IgE-producing plasma cells in *H. pylori*-infected gastric mucosa (*Berczi et al. 2000*). Elevated total IgE levels have been observed in *H. pylori*-associated gastritis and ulcer and specific anti-*H. pylori* antibodies bound to basophil leukocytes have been identified in the sera of seropositive patients (*Aceti et al. 1991*). Specific IgE takes part in local inflammation via the degranulation of mast cells caused by subsequent antigen stimuli. Patients infected with *H. pylori* show elevated levels of eosinophil cationic protein, eosinophil cationic factor and major basic factor, which is a consequence of local eosinophilia (*Berczi et al. 2000*). Eosinophils could also serve as a direct target due to their low-affinity IgE receptors. IgE and eosinophilia may represent a normal inflammatory reaction in part of the late-phase type I immune response against *H. pylori (Fekete 1998)*. Nevertheless, *H. pylori*-specific IgE is not common in CU (*Liutu et al. 1998*), and there might not be a link between *H. pylori* and CU. However, a modulatory action of *H. pylori* on histamine release from mast cells and basophils has been established (*Lutton et al. 1995*).

Despite early immune responses, *H. pylori* infection is associated mainly with the Th1 response that is responsible for the epithelial damage and gastroduodenal morphologic alterations. Hungarian authors recently demonstrated enhanced lymphocyte reactivity to *H. pylori* in CU (*Hídvégi et al. 2001*). In CU, there could be an increased cellular reactivity as a consequence of underlying continuous autoantigen stimulation (e.g. FcεRIα or antithyroid peroxidase [TPO]), which would be enhanced by *H. pylori*-induced lymphocyte responsiveness.

Complement activation takes part in host reactivity against *H. pylori*. In the absence of complement, marked amounts of bacteria remain extracellularly attached. If complement is present, internalization and morphological destruction are significantly enhanced. Coating the bacteria with IgG and IgM results in complement activation liberating C5a (*Mollenkopf et al 1990*). *H. pylori* and its lipopolysaccharides engage in the classical activation pathway. Complement activaton and consecutive consumption by antibodies directed against *H. pylori* are so excessive that they result in acute episodes of hereditary angioneurotic oedema (*Farkas et al. 2001*). Complement activation and circulating anaphylatoxins (C5a and C3a) have an enhancing effect on mast-cell degranulation. "Focal" infection can activate complement, leading to the allergic reactions frequently seen in everyday dermatological practice and in the pathogenesis of urticaria (*Bakos et al. 1990; 1995*). By liberating C5a anaphylatoxins, *H. pylori* infection might costimulate effects of FcεRIα on skin mast cells, and could henceplay a trigger role in autoimmune urticaria.

H. pylori colonizes the gastric mucosa and leads to gastric mucosal damage. It can alter the structure and the mucus composition, and a decrease of the epithelial barrier integrity could lead to an increased absorption. This increased permeability to antigens could be responsible for allergic sensitization to food antigens. Specific IgE against food antigens has been demonstrated in connection with *H. pylori* in patients with ulcer (*DeLazzari 1989*). A number of studies have reported an association of *H. pylori* infection with food allergy (*Corrado et al. 1998, Figura et al. 1999*). We confirmed a correlation between the presence of food-specific IgE and *H. pylori* infection in CU in one study (*Bakos 2002*).

A sensitization to food antigens due to *H. pylori*-caused enhanced absorption has also been implicated in the pathogenesis of CU.

The resolution of chronic inflammation in *H. pylori* gastritis has been demonstrated to be a slow process, the healing requiring several months (*Valle et al. 1991*). This finding explains the observation that the healing or improvement of CU occurred within 3-12 weeks after successful eradication treatment (see in *Chapter 1*).

Despite conflicting results of eradication therapy, *H. pylori* may have an indirect role in urticaria (*Greaves 2001*). The positive correlation between a positive autologous test and *H. pylori* (*Hizal et al. 2000*) suggests its role in the autoimmune pathogenesis of urticaria.
I found a characteristic difference in prevalence of *H. pylori* in autoimmune urticaria with coexistent autoimmune thyroiditis as compared with that without thyroid autoimmunity (see in *Chapter 2*).

The evidence suggests that bacterial proteins could elicit autoimmunity as a consequence of molecular similarity to human antigens. *H. pylori* may play a part in triggering autoimmunity, possibly as a result of the high degree of sequence homology exhibited by the heat shock protein 60 (hsp60) family (*Barton et al. 1998*), although other factors such as cross-reactivity between Lewis X and Y antigens and bacterial lipopolysaccharide may also be important (*Aspinall et al. 1996*). Autoantibodies against hsp60 perpetuate mucosal permeability via damage to the gastric epithelial cells (*Barton et al. 1998*).

Lipoprotein 20 (lpp20) is an outer membrane protein. The outer membrane is a continuous structure on the surface of Gram-negative bacteria. *H. pylori* may shed part of its outer membrane as vesicles when the cells are growing. They could then be released into the extracellular space and enter the gastric mucosa. The extracellular release of soluble *H. pylori* proteins may have important functional consequences, including a potential role in inciting a gastric mucosal inflammatory response (*Mai et al. 1991*). They are of particular significance as a potential target for protective immunity (*Keenan et al. 2000b*). The 19 kD band represents lpp20, which has a classical lipoprotein signal sequence (*Kostrzynska et al. 1994*). Lipoproteins have a structural similarity to oxidized low-density lipoprotein, so they could elicit autoimmune reactivity (*Vaarala et al. 1993*).

We previously found that the prevalence of anti-*H. pylori*-associated lpp20 antibody, analysed by Western blot, was considerably higher in *H. pylori*-positive patients with CU than in *H. pylori*-positive patients without it (*Bakos et al. 2000*). In order to assess the

occurrence of *H. pylori* infection in CU, we extended our preliminary observation of the high prevalence of anti-lpp20 antibodies in *H. pylori*-positive patients with CU. (see in *Chapter 3*)

Chapter 1

Effects of *Helicobacter pylori* eradication on chronic urticaria

1.1. Patients and methods

A total of 36 chronic idiopathic urticaria patients were enrolled in this study. The patients were examined at the Department of Dermatology, Hetényi Hospital, Szolnok during 1996-97. They were investigated according to a previous study (*Bakos et al. 1995*). Patients with urticarial vasculitis, hereditary angioedema and physical urticaria were excluded from the study.

Routine diagnostic approach

The patients underwent a full investigation of the history, physical examination, consultation with the gynaecologist, urologist, dentist and ENT specialist, dental and sinus radiography, abdominal ultrasonography and laboratory tests: eythrocyte sedimentation rate, complete blood counts, urinanalysis, liver and kidney functions, hepatitis serology, antinuclear antibody (Hep2 cell, indirect immunofluorescence), anti-SSa, anti-SSb, complement activation and stool examination. Intradermal testing was performed with a mixture of grasses, tree pollens, ragweed, cereals, mugwort, housedust mite, cat hair, dog hair, cow milk, egg, peanut, nuts and wheat (Allergopharma). Epicutan tests (European standard, Epipharm) were performed. Total IgE was investigated by IgE Quick.

Determination of *H. pylori* infection

All patients were subjected to oesophagogastroduodenoscopy, and biopsies were taken from the gastric antrum and corpus. The presence of *H. pylori* was asessed by Giemsa staining and by a rapid urease test. *H. pylori*-specific IgG determination was performed by RIDA®(R-Biopharm GmBH, Darmstadt, Germany).

Treatment and follow-up of *H. pylori* infection

Patients who were infected with *H. pylori* were given triple antimicrobial treatment consisting of omeprazole 2x20 mg daily in the first week (1x 20 mg daily in the second week), 2x500 mg clarithromycin daily and 2x1000 mg amoxycillin daily for 2 weeks. During the eradication, concomitant treatment with the non-sedating antihistamins loratadine (10 mg daily) was allowed for patients with CU symptoms.

Four weeks after the completion of therapy, the effectiveness of eradication was assessed by gastroscopy, urease tests and serum tests for circulating *H. pylori* antibodies. The effect of the treatment on urticaria was evaluated via a symptom score system (0-3 score) during 12 weeks.

Statistical methods

Fisher's exact test was applied to compare the prevalence of *H. pylori* infection in the study groups and the normal population. Analysis of variance between the values of IgE was estimated with the Mann-Whitney test. A probability level $p \leq 0.05$ was considered statistically significant.

1.2. Results

Examinations were performed on 36 patients with CU: 24 women and 12 men aged between 20 and 74 years (mean 41.3 ± 16.4). None of them had CU of known etiology, e.g. foci, food allergy, intolerance reaction or contact allergy with the exception of possible infection with *H. pylori*. Gastroscopy did not reveal any morphologic changes in the patients. The urease test was positive in 25 of the 36 (69%). 11 of the 36 patients remained idiopathic after investigation for *H. pylori*. *H. pylori* infection was detected in biopsy specimens of these patients. Urease testing and direct staining of biopsy specimens indicated that the "negative" patients did not have morphologic disturbances or *H. pylori* colonization. *H. pylori*-associated gastritis was diagnosed by histopathologic examination in the patients infected by the bacterium, although it was mild.

There was a higher prevalence of *H. pylori* seropositivity in younger (aged 20-34) and middle-aged (aged 45-55) patients with CU as compared with previously published results from a representative sample of the Hungarian population (*Tamássy et al. 1995*), whereas the age distribution of the studied individuals did not differ significantly (*Figure 1.1*).

The serum total IgE was elevated in 19 of the 25 *H. pylori*-infected patients, but in none of the *H. pylori*-negative patients (176.4 kU/L *vs.* 46 kU/L) ($p<0.03$).

All the *H. pylori*-infected patients received eradication therapy. Control gastroscopy performed after 4 weeks revealed the presence of the bacterium in only 1 case; a second

course of treatment proved necessary. Successful eradication was confirmed by histology and by urease testing in 24 patients. *H. pylori*-specific IgG was positive in all 24 patients.

In 6 patients the CU was eliminated 2 weeks after eradication (follow-up 4 weeks), while in 8 patients it was improved significantly. Six weeks after eradication (follow-up 8 weeks) complete remission was observed in 11 patients, and partial remission in 6, whereas at the end of the follow-up (12 weeks) there were 18 cases of complete (72%), and 4 of partial remission (16%). Eradication was not beneficial for the CU in 3 patients (12%). Taken together, a large majority (88%) of the patients who underwent *H. pylori* eradication therapy exhibited the disappearance or improvement of the CU (*Figure 1.2)*. In contrast, among the seronegative patients with CU (n=11), only 3 (27.2%) showed a significant improvement (a significant reduction of the CU symptoms and/or the use of antihistamines) and 2 (18.1%) a spontaneous remission within 12 weeks.

1.3. Discussion

H. pylori infection is one of the most frequent human infections. In this study we found a similar prevalence in CU relative to the normal population. There was a significant difference in the course of the symptoms in seropositive CU patients after eradication therapy as compared with seronegative patients with CU. In 88% of the seropositive patients, the CU symptoms disappeared or improved after the eradication treatment, whereas the condition improved spontaneously in only 45.3% of the seronegative subjects. This is similar to the situation for untreated *H. pylori* seropositive patients (50%) (*Wedi et al. 1998*), which provides evidence for a causal relation between *H. pylori* gastritis and CU.

The total IgE level was significantly higher in the seropositive patients than in the seronegative group. Other authors have shown that one-fifth of the patients present elevated total IgE serum levels (*DeLazzari et al. 1994*).

During viral or bacterial infections of the digestive tract, the intestinal permeability to food antigens generally increases, because of the alterations caused in the gastrointestinal epithelium by infectious agents and by the inflammatory reaction. In the context of such an inflammatory environment, the local antigen-presenting cells (mainly dendritic cells) switch from a tolerogenic to an immunogenic state. This process favours the development of an immune response instead of the normal suppressive response, which is the basis of oral

tolerance. The bacterial and viral stimuli could also modify the intestinal permeability to food antigens due to the alteredbarrier function of the epithelium, or could activate the costimulatory molecules at the surface of local antigen-presenting cells. However, the increased intestinal transport of macromolecules does not lead systematically to an increased allergic sensitization. Not only a genetic susceptibility plays a role in the development of allergic responses to food antigens absorbed in the intestine, but also other factors that interfere with antigen presentation, such as the type of antigen, the type and status of the antigen-presenting cell, the presence of bacterial adjuvants, the expression of costimulatory molecules at the time of presentation, or the cytokines present during T-cell activation.

The bacterial intestinal microflora plays an important role in the immune responses to luminal antigens. Nonpathogenic bacteria (probiotics) have recently been shown to attenuate the synthesis of proinflammatory effector molecules (NFκB) elicited by diverse proinflammatory stimuli, including pathogenic bacteria (*Neish et al. 2000*). The mechanism of such an inhibitory effect consists in the blockade of inhibitory κβ–α degradation, preventing subsequent nuclear translocation of the active NFκB dimer and then the transcription of genes coding for inflammatory cytokines.

H. pylori colonization induces a strong inflammatory response in the gastroduodenal mucosa, with a consecutive enhanced permeability of the gastric mucosa. The gastric epithelium, like the small intestine epithelium, is able to absorb small amounts of macromolecules, and this antigen absorption may induce the IgE-mediated sensitivity reactions to these antigens (*Matysiak-Budnik et Heyman 2002*). The stomach is considered a major site of involvement in food-induced hypersensitivity reactions. A marked increase in the number of IgE-producing cells in the gastric mucosa has been observed in patients with peptic ulcers infected by *H. pylori* (*Berczi et al. 2000*). Significantly higher total IgE serum levels were found in patients with peptic ulcers than in healthy subjects (*De Lazzari et al. 1994*)

It has recently been reported that inflammation and enhanced permeability in *H. pylori* gastritis directly stimulates IgE production in response to the most common alimentary allergens, even in patients without clinical evidence of allergy (*Figura et al. 1999*). Specific IgE induced by food and inhalant allergens has been found in 28% of peptic ulcer patients and 4% of controls (*De Lazzari et al. 1989*).

A number of data support the possible association between *H. pylori* and food allergy. Bacteria can colonize the gastric mucosa and alter the gastric barrier. All the bacterial factors, and also the inflammatory mediators, may lead to gastric mucosal damage. Increased gastric permeability to sucrose has been found in patients infected with *H. pylori* (*Fucuda et al. 2001).* The experimental data indicate that *H. pylori* can increase the passage of intact antigens across the epithelial barrier (*Matysiak-Budnik et Heyman 2002*).

It has been demonstrated that there is a higher percentage of infection with this pathogen among food-allergic children than among the control group (*Corrado et al. 1998*). We have observed a relationship between sensitization to food and *H. pylori.* The prevalence of food-specific IgE was 41.4% in CU patients with *vs.* 7.1% in patients without *H. pylori* infection (*Bakos et Szemere 2002*).

Previous data indicated that healing or improvement occurred within 3-12 weeks after eradication therapy (*Table 1.1*).

The absorption of vasoactive ingredients and the passage of food components through the barrier continue long after eradication because the polymorphonuclear infiltration and epithelial damage diminish only after some period. On the other hand, food allergy increases the intensity of gastric inflammation, which leads to the persistence of gastric inflammation for months and even years after eradication of the bacterium.

These data strengthen the hypothesis that in some subjects, chronic infection with *H. pylori* may play a role in the development of allergic sensitization to food antigens.

Figure 1.1. Comparison of data obtained from a representative sample of the Hungarian population (*Tamássy et al. 1995*) with present data on CU patients (n=36)

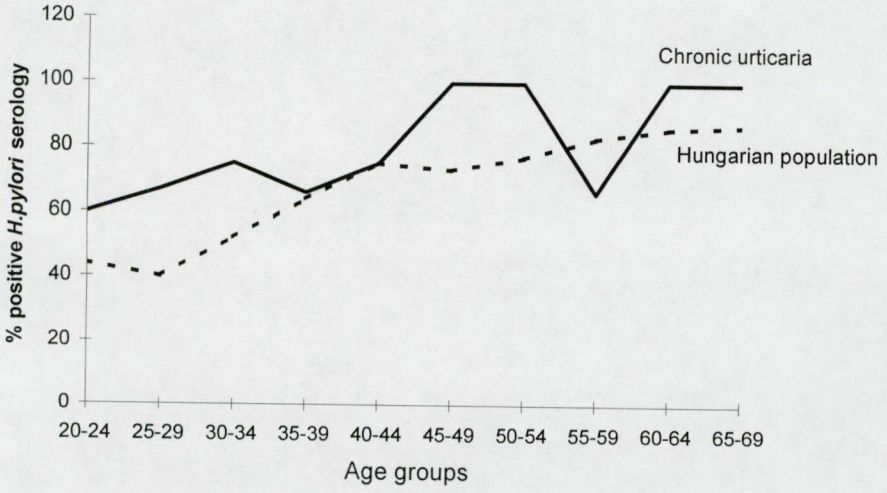


Figure 1.2. Course of CU after eradication therapy

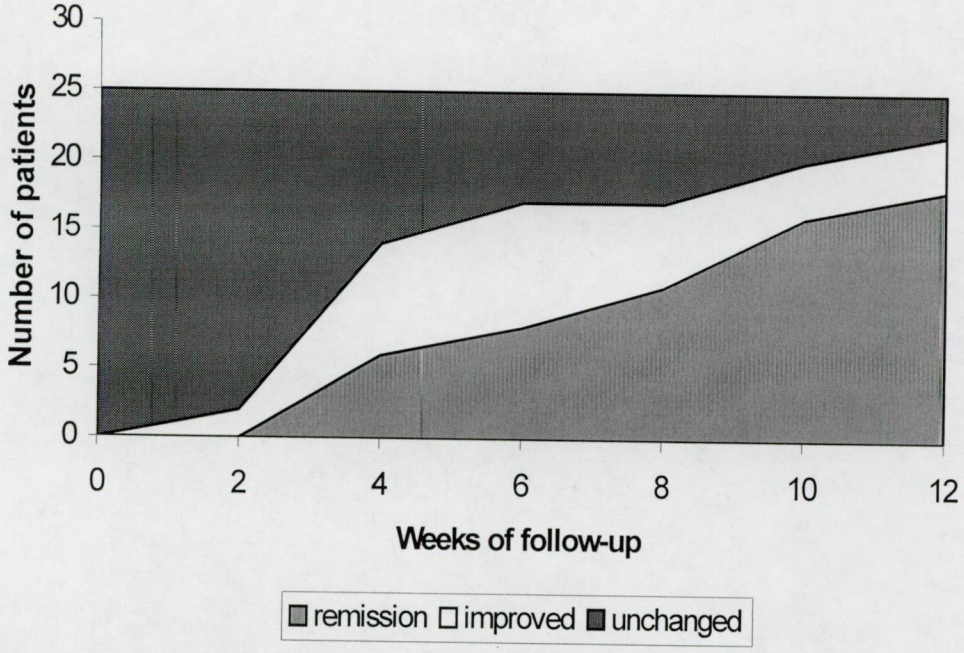


Table 1.1. Studies relating to the frequency of *H. pylori* infection in CU and the effectiveness of eradication therapy

Reference	*H.pylori*-positive cases	Eradicated	Follow-up period (weeks)	Course of CU after eradication
Kalas et al.(1996)	17/40	17	8-12	Significant improvement
Tebbe et al. (1996)	14/25	14	8-10	6 PR, 8 CR
Bohmeyer et al. (1996)	8/10	8	4	CR
Wedi et al (1998)	47/100	21	12	14 CR, 5 PR
Bakos et al. (1998)	25/36	25	12	4 PR, 18 CR

PR: partial remission, CR: complete remission, CU chronic urticaria

Chapter 2.

Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria (effects of *Helicobacter pylori* on pathogenesis of autoimmune urticaria)

2.1. Patients and methods

Patients

Forty-eight patients (30 women and 18 men, aged 14 to 75 years; mean: 40.8 ± 16.4 years) with CU were investigated. They had no food allergy or intolerance, contact allergy, bacterial focus or parasitic infection, physical urticaria, urticarial vasculitis, hypocomplementaemic urticaria or polysystemic autoimmune diseases. The duration of the symptoms ranged between 2 and 240 months (mean 18.4 ± 19.1).

Autologous serum test

0.1 mL of autologous serum and autologous serum diluted 1:1 with physiological saline were administered to the patients' forearms (flexor sides). The reaction was examined 30 minutes later. A wheal with a diameter at least 1.5 mm greater than that of a control wheal induced with saline solution was accepted as positive (*Greaves 2000*).

Thyroid function and autoantibodies

Thyroxine (free T4) and thyroid-stimulating hormone (TSH), antithyroid peroxidase antibody (TPO) and antithyroglobulin antibody (TG) levels were investigated. Free T4 and TSH were measured by Microparticle Enzyme Immunoassay (MEIA) (Abbott Laboratories, USA), and TG and TPO by sequential immunometric assay with an IMMULITE Analyser (DPC, United Kingdom).

Determination of *H. pylori* infection

All patients underwent gastroscopy and urease testing. They had no dyspeptic symptoms in their history or during the observation period and only mild gastritis was seen on endoscopy. *H. pylori*-specific IgG determination and immunoblotting for the detection of antibodies (IgG) against *H. pylori* were performed by RIDA® (R-Biopharm GmBH, Darmstadt, Germany) with the following molecular weights: 120 kD cytotoxicity associated gene A (CagA), 87 kD (VacA), 67 kD (OMP 67), 62 kD (urease B), 58 kD (hsp), 54 kD

(flagellin), 47 kD, 33 kD, 29 kD (urease H), 28 kD (hcpA - *H. pylori* cysteine-rich protein A), 25 kD (urease D) and 19 kD.

Statistical methods

Fisher's exact test was applied to compare the prevalence of thyroid autoimmunity and *H. pylori* infection in the two study groups. Analysis of variance between the values of basophil cells and IgE was performed with the Mann-Whitney test. A probability level $p \leq 0.05$ was considered statistically significant.

2.2. Results

The 48 patients with CU were divided into two subgroups on the basis of the results of autologous serum tests. The autologous serum test was positive in 26 patients (54.2%): 16 women and 10 men (*autoimmune group*). The autologous serum test was negative in 22 patients (45.8%): 13 women and 9 men (*non-autoimmune group*).

The basophil cell count was 0.030 ± 0.012 10^9/L in the autoimmune group, and 0.046 ± 0.011 10^9/L in the non-autoimmune group; the difference was significant: $p = 0.001$. The IgE level was 175.7 ± 196.7 IU/mL in the autoimmune group, and 213.2 ± 237.9 IU/mL in the non-autoimmune group, but the difference was not significant (*Table 2.1*). We found no difference in the prevalence of *H. pylori* infection between the two groups. On the basis of the urease test, the histology and the *H. pylori*-specific IgG, 17 of the 26 autoimmune cases (65.4%) and 13 of the 22 non-autoimmune cases (59.1%) were infected with *H. pylori*.

The prevalence of antithyroid antibodies was different in the two groups of CU patients. In the autoimmune group, we found 11 patients (42.3%) with TPO, whereas in the non-autoimmune group, there were 3 patients (13.6%) with TPO. The difference in prevalence of TPO between the two groups was significant ($p = 0.03$). None of the patients had known of the thyroid disease previously. The prevalence of TG did not differ significantly in two groups (4 *vs.* 1 patients; 18.2% *vs.* 3.8%). Two patients from the autoimmune group with an elevated TPO had a lowserum TSH level; accordingly, the condition was considered to be subclinical hyperthyroidism. Both patients were examined by an endocrinologist.

In a further analysis of the autoimmune patients with TPO, we found differences in frequency of *H. pylori* infection as compared with the autoimmune patients without TPO (*Table 2.2*). We found 10 patients with TPO infected with *H. pylori* (90.9%) in the

autoimmune group, only 1 patient in this group being non-infected. Of the 15 autoimmune patients without TPO, 7 (46.7%) were infected with *H. pylori*. The difference in the prevalence of *H. pylori* infection was significant between autoimmune urticaria with and without thyroid autoimmunity ($p=0.02$).

We investigated *H. pylori*-specific IgG in all 17 infected patients in the autoimmune group (with and without TPO). We found antibody to 120 kD in 14 patients (82.4%), to 87 kD in 16 (94.1%), to 67 kD in 16 (94.1%), to 62 kD in 6 (35.3%), to 58 kD in 17 (100%), to 54 kD in 14 (82.4%), to 47 kD in 8 (47.1%), to 44 kD in 10 (58.8%), to 33 kD in 9 (52.9%), to 29 kD in 12 (70.6%), to 28 kD in 16 (94.1%), to 25 kD in 13 (76.5%), and to 19 kD in 16 (94.1%). The *H. pylori*-specific IgG antibodies revealed a similar prevalence in the subjects with TPO (10 patients) and in those without TPO (7 patients), except for 120 kD CagA: (patients with *vs.* without TPO: 120 kD: 10 *vs.* 4; 87 kD 9 *vs.* 7; 67 kD: 10 *vs.* 6; 62 kD: 4 *vs.* 2; 58 kD: 10 *vs.* 7; 54 kD: 8 *vs.* 6; 47 kD: 5 *vs.* 3; 44 kD: 5 *vs.* 4; 33 kD: 5 *vs.* 4; 29 kD: 7 *vs.* 5; 28 kD: 9 *vs.* 7; 25 kD: 8 *vs.* 5 and 19 kD: 9 *vs.* 7.) The difference between the patients with and without thyroid autoimmunity was significant only in the case of 120 kD ($p < 0.05$).

2.3. Discussion

The clinical diagnosis of autoimmune urticaria is currently based on autologous serum skin testing. We distinguish two groups of CU patients on the basis of the result of the autologous skin test. We found no laboratory differences between the autoimmune and non-autoimmune groups except for the basophil count, in accordance with the previous data (*Greaves 2000*).

We observed a relationship between autoimmune urticaria and autoimmune thyroiditis. A positive autologous serum skin test was previously described in patients with autoimmune urticaria associated with thyroid autoimmunity (*Gaig et al. 2000*).

We found a high prevalence of thyroid autoimmunity among the CU patients, though all but 2 cases of subclinical hypothyroidism would have remained unrecognized without investigation of the thyroid autoantibodies. Despite the high titres of thyroid autoantibodies, most patients remain asymptomatic for years and have a normal thyroid function; ultimately,

however, a substantial proportion of these patients develop overt hypothyroidism (*Leznoff et al. 1983*). Our results suggest that a complete thyroid examination with hormone and autoantibody assays should be performed, because examination of the thyroid function alone is insufficient in CU.

In the present study, we found a characteristic difference in theprevalence of *H. pylori* in autoimmune urticaria with coexistent autoimmune thyroiditis as compared with that without thyroid autoimmunity. A high seroprevalence of *H. pylori* infection was earlier observed in patients with autoimmune thyroiditis (*Figura et al. 1999*).

There is growing evidence that the phenomenon of parasite-host mimicry may initiate or maintain autoimmunity. Abundant data point to the presence of cellular and humoral autoimmune responses in patients with *H. pylori* infection (*Realdi et al. 1999*). In a mechanism known as antigen mimicry, highly conserved immunologic molecules expressed by infectious pathogens may act as a trigger for the immune responses that cross-react with the host cellular antigens. A great number of cross-reacting antibodies take part in the pathogenesis of the stomach and duodenal alterations caused by *H. pylori*, e.g. antibodies against *H. pylori* cross-react with antral mucosal cells (the membrane of the secretory canalicular structures of the parietal cells) and gastrin-producing cells. Antigastric antibodies play a role in the pathogenesis of gastric mucosal atrophy and gastritis (*Negrini et al. 1997*). Type A gastritis (corpus-restricted autoimmune gastritis) is often associated with pernicious anaemia, an autoimmune disease.

Various findings have previously been published suggesting an association between *H. pylori* infection and some extradigestive autoimmune ailments, such as Sjögren's syndrome (*Figura et al. 1994*), scleroderma (*Kalabay et al. 2002*), Henoch-Schönlein purpura (*Reinauer et al. 1995*) and thyroiditis (*Figura et al. 1999*). The basic mechanism might be antigen mimicry between *H. pylori* antigens and several essential human antigens, as other infective agents can introduce or participate in an autoimmune pathogenesis.

We found a connection between autoimmune thyroiditis and CagA + *H. pylori* strains. Monoclonal antibodies to a *H. pylori* strain with CagA positivity reacted with follicular cells of the thyroid gland, and the *H. pylori* organism possessing the CagA pathogenicity island carries a gene encoding for an endogenous peroxidase. There is a

molecular similarity between human thyroid peroxidase and *H. pylori* peroxidase; this causes immunologic cross-reactivity and might be a possible factor in the development of autoimmune thyroid disease (*Figura et al. 1999*).

A positive correlation has been described between *H. pylori* infection and the result of the autologous serum skin test (*Hizal et al. 2000*).

Our findings suggest the possibility of the triggering of cross-reactivity between CagA+ *H. pylori* strains and two connected autoimmune disorders, autoimmune thyroiditis and autoimmune urticaria. This indicates an indirect aetiological role of *H. pylori* in triggering autoimmune urticaria in at least a selected group of patients.

Table 2.1. Characteristics of patients with chronic autoimmune urticaria and chronic non-autoimmune urticaria

Characteristics	Autoimmune (n= 26)	Non-autoimmune (n= 22)	p
Age	46.5 ± 18.6	36.1 ± 18.9	0.03#
Basophil cell count (G/L)	0.030 ± 0.012	0.046 ± 0.011	0.001#
Total IgE (IU/mL)	175.7 ± 196.7	213.2 ± 237.9	NS
Infected with *H. pylori* (n / %)	17 (65.4%)	13 (59.1%)	NS
TPO Ab (n / %)	11 (42.3%)	3 (13.6%)	0.03*
TG Ab (n / %)	4 (18.2%)	1 (3.8%)	NS

Mann-Whitney test

*Fisher's exact test

Table 2.2. Survey of thyroid autoimmunity in patients with chronic autoimmune urticaria (n = 26)

Characteristics	With TPO (n=11)	Without TPO (n=15)
Infected with *H. pylori*	10 (90.9%)	7 (46.7%)
Without *H. pylori*	1 (9.1%)	8 (53.3%)

Fisher's exact test: p=0.024

Chapter 3

High prevalence of IgG and IgA antibodies to 19 kD *Helicobacter pylori*-associated lipoprotein in chronic urticaria

3.1. Patients and methods

Fifty-six patients (34 women and 22 men, 14 to 75 years of age; mean ± S.D.: 40.8 ± 16.4 years) with CU were investigated. The duration of the disease varied between 2 and 240 months (median: 6 months, IQ range: 3.5-18.0). They had no other underlying causes of urticaria, such as food allergy or intolerance, contact allergy, bacterial focus or parasitic infection, physical urticaria, urticarial vasculitis, hypocomplementemic urticaria or polysystemic autoimmune diseases. They had no dyspeptic symptoms in their history or during the observation period.

The control group comprised a selected group of 33 *H. pylori*-positive, severely dyspeptic, but non-urticarial patients (12 men and 21 women, 24 to 75 years of age, mean: 51.1 ± 13.3 years) with high grade histologically proven *H. pylori*-associated gastritis. All patients underwent gastroscopy, histology of the gastric mucosa and urease testing. The degree of gastritis was estimated by means of the Updated Sydney System.

Anti-*H. pylori*-specific IgG and IgA were determined by the RIDA® test (R-Biopharm GmbH, Darmstadt, Germany). Titres of ≥10 IU/L were considered positive, according to the values given by the manufacturer. Immunoblotting was performed with the RIDA® test (R-Biopharm GmbH, Darmstadt, Germany), with the following molecular weights: 120 kD (CagA), 87 kD (VacA), 67 kD (OMP 67), 62 kD (urease B), 58 kD (hsp), 54 kD (flagellin), 47 kD, 33 kD, 29 kD (urease H), 28 kD (hcpA), 25 kD (urease D) and 19 kD (lpp20). In the test are anti-human-IgG and IgA conjugates (IgG antibodies from rabbit conjugated with peroxidase).

Fisher's exact test was applied to compare the anti-*H. pylori* antibody profiles and gastritis scores in the two study groups. Analysis of variance between the values of *H. pylori*-specific IgG and IgA was performed with the Mann-Whitney test. A probability level $p \leq 0.05$ was considered statistically significant.

3.2. Results

H. pylori-specific IgG and IgA titres in patients with and without CU

The 56 patients with CU were divided into two subgroups on the basis of the findings of gastroscopy and the urease test. Subgroup 1 comprised 33 urease-positive patients with mild gastritis on histology (score 1.3), while 23 urease-negative, non-gastritic patients formed subgroup 2 (*Table 3.1*). They were categorized as "idiopathic" urticarial patients.

Whereas the *H. pylori*-specific IgG level was elevated (≥ 10 IU/L) in each patient in subgroup 1, all the patients in subgroup 2 remained seronegative. In each patient in the control group, endoscopy and the histology demonstrated severe gastritis (score 3.8) and the *H. pylori*-specific IgG proved positive. The gastritis score was significantly higher in the control group than in subgroup 1 (*Table 3.1*).

The *H. pylori*-specific IgA was positive in 13 of the 33 patients in subgroup 1. In subgroup 2, the anti-*H. pylori* IgA levels were not elevated. In the control group, high anti-*H. pylori* IgA levels were detected in 28 of the 33 patients (84.8%). The titres of anti-*H. pylori* IgG and IgA antibodies were significantly higher in the control group than in subgroup 1 ($p = 0.0128$ and $p<0.0001$) (*Table 3.1*).

Patients with and without CU exhibit different IgG and IgA profiles against *H. pylori* antigens

Western blot analysis was performed on 33 *H. pylori*-positive patients with CU (subgroup 1) and in the control group. In subgroup 2, the low specific IgG or IgA (0-10 IU/L) did not furnish measurable values on Western blot analysis; accordingly, these patients were not included in the Western blot study. We observed a surprisingly higher prevalence of anti-lpp20 IgG antibodies (93.9%) in subgroup 1 than in the control group (21.2%, $p < 0.0001$) (*Table 3.2*). The IgG levels to other *H. pylori* antigens did not indicate any significant difference between these two groups (*Fig. 3.1*).

Immunoblotting for IgA antibodies against *H. pylori* antigen determinants was performed in 13 *H. pylori*-positive patients with CU (all patients in subgroup 1 with elevated *H. pylori*-specific IgA) and in 16 control patients. There was a significant difference in the prevalence of anti-lpp20 IgA antibodies between the CU and the non-CU patients (46.1% *vs.*

6.3%, p = 0.0029) (*Table 3.3*). There were no significant differences between the two groups as regards the prevalence of other anti-*H. pylori* IgA antibodies.

3.3. Discussion

We observed a characteristic difference in humoral immunoreactivity to *H. pylori*-associated lpp20 between *H. pylori*-infected patients with CU, and *H. pylori*-positive patients without CU. As concerns the other *H-pylori*-associated antigen moieties, we found practically identical antibody patterns in the patients with and those without CU.

The 19 kD band represents lpp20, a conserved *H. pylori*-associated lipoprotein that contains a classical lipoprotein signal sequence (*Kostryzinska et al. 1994*). Like many other Gram-negative bacteria, in some special conditions *H. pylori* may shed part of its highly antigenic outer membrane as vesicles, resulting in portions of the membrane blebbing off the surface of growing cells (*Keenan et al. 2000b*). They could then be released into the extracellular space and enter the gastric mucosa. This phenomenon may have important functional consequences, including a potential role in inciting a gastric mucosal response (*Mai et al. 1991, Cao et al. 1998*). Bacterial lipoproteins are not only targets of an immune response, but also act as immunostimulatory molecules (*Haupl et al. 1997*). They may play a role in the pathogenesis of several autoimmune diseases, e.g. rheumatoid arthritis and systemic lupus erythematosus (*Vaarala et al. 1993*). Furthermore, the 19 kD lpp20 may act as a protective antigen, but this protection depends on the magnitude and subclass of the response; for example, an IgG1 subclass monoclonal antibody raised against *H. pylori* lpp20 can reduce or even prevent *H. pylori* colonization (*Keenan et al. 2000a*). This protective antibody response may have a functional role in the prevention or mitigation of gastritis associated with *H. pylori* infection.

Our CU patients had no dyspeptic symptoms in their history or during the observation period, and only mild gastritis was seen on endoscopy. This phenomenon may be explained by the protective effect of anti-lpp20 IgG antibody. On the other hand, people harbouring *H. pylori* with zero or low levels of anti-lpp20 may develop severe gastritis with overt dyspeptic symptoms, as observed in our selected dyspeptic control group.

Our findings suggest that IgG and (in part) IgA antibodies to *H. pylori*-associated lpp20 (in addition to their putative gastroprotective effect) could act as a source of

autoimmunity, and may play a role in the pathogenesis of a subtype of CU, presumably via cross-reactivity between the bacterial lpp20 and some skin antigen components. Further investigation is needed to establish cross-reactivity between lpp20 and skin antigens.

Table 3.1. Titres of *H. pylori*-specific IgG and IgA in patients with and without CU

	Subgroup 1 (CU with *H. pylori* infection)	**Subgroup 2** (CU without *H. pylori* infection)	**Control group** (*H. pylori*-associated gastritis without CU)
No. of patients	33	23	33
Histologic score *p	1.3	0	3.8
***H. pylori*-specific IgG**			
No. of patients ≥ 10 IU/L	33 (100%)	0	33 (100%)
Median **p, +p	48.0 IU/L	3.6 IU/L	76.2 IU/L
IQ range	23.8 - 116.6 IU/L	0 - 5.9 IU/L	41.3 - 112.6 IU/L
*p = 0.001 between subgroup 1 and control group ** p <0.0001 between subgroup 1 and subgroup 2 + p = 0.0128 between subgroup 1 and control group, compared by the Mann-Whitney test.			
***H. pylori*-specific IgA**			
No. of patients ≥ 10 IU/L	13 (39.4%)	0	28 (84.8%)
Median *p, +p	11.9 IU/L	2.4 IU/L	16.5 IU/L
IQ range	3.8 - 22.7 IU/L	0 - 4.0 IU/L	8.0 - 22.5 IU/L
* p = 0.0035 between subgroup 1 and subgroup 2 + p <0.0001 between subgroup 1 and control group, compared by the Mann-Whitney test			

Table 3.2. Western blot analysis of IgG profile against *H. pylori* in CU and control group.

***H. pylori* antigens**	**Subgroup 1** (CU with *H. pylori* infection) (n = 33)	**Control group** (*H. pylori*-associated gastritis without CU) (n = 33)
120 kD	28# (84.8%)	33# (100%)
87 kD	24 (72.7%)	28 (84.8%)
67 kD	26 (78.8%)	27 (81.8%)
62 kD	12 (36.4%)	21 (63.6%)
58 kD	33 (100%)	33 (100%)
54 kD	27 (81.8%)	22 (66.7%)
47 kD	15 (45.5%)	19 (57.6%)
44 kD	19 (57.6%)	18 (54.5%)
33 kD	20 (60.6%)	12 36.4%)
29 kD	23 (69.7%)	20 (60.6%)
28 kD	31 (93.9%)	32 (96.9%)
25 kD	26 (78.8%)	22 (66.7%)
19 kD	31 (93.9%)*	7 (21.2%)

number of positive patients.

* $p < 0.0001$ compared to control group, Fisher's exact test

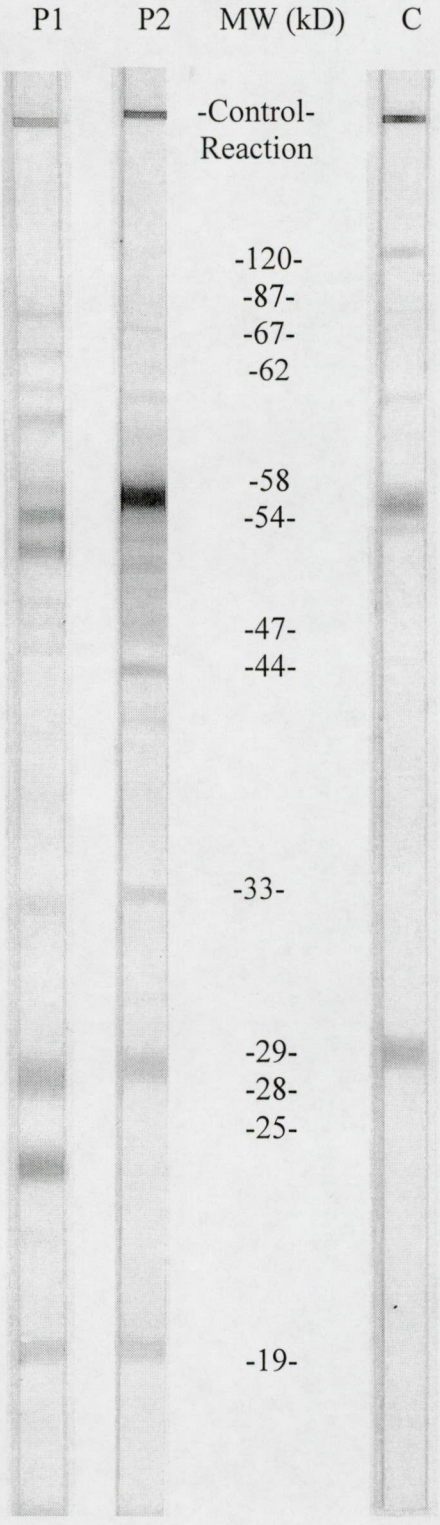
Table 3.3. Western blot analysis of IgA profile against *H. pylori* in CU and in the control group

***H. pylori* antigens**	**Subgroup 1** (CU with *H. pylori* infection) (n = 13)	**Control group** (*H. pylori*-associated gastritis without CU) (n = 16)
120 kD	9# (69.2%)	12# (75%)
87 kD	8 (61.5%)	10 (62.5%)
67 kD	5 (38.5%)	8 (50.0%)
62 kD	12 (92.5%)	15 (93.8%)
58 kD	11 (84.6%)	15 (93.8%)
54 kD	6 (46.2%)	6 (37.5%)
47 kD	3 (23.1%)	4 (25.0%)
44 kD	4 (30.7%)	4 (25.0%)
33 kD	1 (7.7%)	2 (12.5%)
29 kD	5 (38.5%)	5 (31.3%)
28 kD	9 (69.2%)	7 (53.8%)
25 kD	2 (15.4%)	3 (18.7%)
19 kD	6 (46.1%)*	1 (6.3%)

number of positive patients.

* $p < 0.0029$ compared to control group, Fisher's exact test

Fig. 1. IgG Western blot results. P1 and P2 are CU patients infected with *H.pylori* and C is a patient from the control group.



References

1. **Aceti** A, Celestino D, Cafferro M, Casale V, Citarda F, Conti EM et al. Basophil-bound and serum immunoglobulin E directed against *Helicobacter pylori* in patients with chronic gastritis. *Gastroenterology* 1991; **101**: 131-137

2. **Aspinall** GO, Monteir MA, Pang H. et al. Lipopolysaccharide of *Helicobacter pylori* type strain NCTC 11637 (ATCC43504): structure of the O antigen chain and core oligosaccharide regions. *Biochemistry* 1996; **35**: 2489-2497.

3. **Bakos** N, Mészáros Cs, Debreczeni M: A chronicus urticaria vizsgálata. *Orvosi Hetilap* 1990, **131**: 1183-1186.

4. **Bakos** N, Mészáros Cs, Hunyadi J: Immunpathológiai vizsgálatok chronicus urticariaban. *Orvosi Hetilap* 1995; **136**: 2603–2608.

5. **Bakos** N, Hillander M, Viczián Á, Fekete B, Füst G. Investigation of chronic urticaria associated with *Helicobacter pylori. Immunol Lett* 2000; 73: 276.

6. **Bakos** N, Szemere P. The role of *Helicobacter pylori* in pathogenesis of chronic urticaria. *Allergy* 2002; **57**: S73, 314.

7. **Barton** SGRG, Winrow VR. Rampton DS, Crabtree JE, Beales ILP, Calam J. Circulating antibodies to the 60-kD heat shock protein (hsp) family in patients with *Helicobacter pylori* infection. *Clin Exp Immunol* 1998; **112**: 490-494.

8. **Becker** H, Meyer M, Paul E. Remission ratio of chronic urticaria – "spontaneous" healing or fault of eradication of *Helicobacter pylori*? *Hautarzt* 1998; **49**: 907-911.

9. **Berczi** L, Sebestyén A, Tamássy K, Kopper L. IgE-containing cells in gastric mucosa with and without *Helicobacter pylori* infection. *Pathol Res Pract* 2000; **196**:

831-834.

10. **Bohmeyer**, J., Heller, A., Hartig, C., Westenberger-Treumann, M., Huchzermeyer, H., Otte, H., Stadler, H.: Assotiation der chronischen Urticaria mit *Helicobacter pylori*-induzierter Antrum-Gastritis. *Hautarzt* 1996, **47**: 106-108

11. **Cao** P, McClain MS, Forsyth MH, Cover TL. Extracellular release of antigenic proteins by *Helicobacter pylori. Infect Immun* 1998; **66**:2984-2986.

12. **Corrado G,** Luzzi I, Lucarelli S. Frediani T, Pacchiarottti C, Cavaliere N, Rea P, Cardi E. Positive association between *Helicobacter pylori* infection and food allergy in children. Scand J Gastroenetol 1998; **33**: 1135-9.

13. **De Lazzari F**, Venturi C, Mancin O et al. Food specific IgE in gastric and duodenal mucosa. *Ital J Gastroenterol* 1989; **21**: 347

14. **De Lazzari F**, Mancin O, Plebani M, Venturi C, Battaglia G, Vianello F, Galliani EA, Di Mario F, Naccarato R. High IgE serum levels and peptic ulcers: clinical and functional approach. *Ital J Gastroenterol* 1994, **26**: 7-11.

15. **Delevaux** I, Andre M, Tridon A, Aumaitre O. Chronic urticaria and Hashimoto-Hashimoto's thyroiditis: report of 6 cases. *Rev Med Interne* 2001; **22**: 232-7

16. **Di Campli** C, Gasbarrini A, Nucera E, et al. Beneficial effects of Helicobacter pylori eradication on idiopathic chronic urticaria. *Dig Dis Sci* 1998; **43**: 1226-9.

17. **Einarsdottir** E, Soderstrom I, Lofgren-Burstrom A et al. The CTLA4 region as a general autoimmunity factor: An extended pedigree provides evidence for synergy with the HLA locus in the etiology of type 1 diabetes mellitus, Hashimoto's thyroiditis and Graves'disease. *Eur J Hum Genet* 2003; **11**: 81-4.

18. **Farkas** H, Fust G, Fekete B, Karadi I, Varga L. Eradication of Helicobacter pylori and improvement of hereditary angioneurotic oedema. *Lancet* 2001; 358: 1695-6.

19. **Fekete** B. Gastroduodenal inflammation associated with *Helicobacter pylori* infection. Diagnostic and therapeutic implication. *Acta Microbiol Immunol Hungarica* 1998; **45**: 175-194.

20. **Ferrer** M, Nakazawa K, Kaplan AP. Complement dependence of histamine release in chronic urticaria. *J Allergy Clin Immunol.* 1999. **104**: 169-172.

21. **Fiebiger** E, Maurer D, Holub H, et al. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995; **96**: 2606-2612.

22. **Fiebiger** E, Hammerschmid F, Stingl G, Maurer D. Anti-FcεRIα autoantibodies in autoimmune-mediated disorders: Identification of a structure-function relationship. *J Clin Invest* 1998, **101**: 243-251.

23. **Figura** N, Giordano N, Burroni D, Macchia G, Vindigni C, Gennari C, Bayeli PF. Sjögren's syndrome and *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1994; **6**: 321-322.

24. **Figura** N. Perrona A, Gennari C, Orlandini G, Giannace R, Lenzi C, Vagliasindi M, Bianciardi L, Rottoli P. CagA-positive *Helicobacter pylori* infection may increase the risk of food allergy development. *J Physiol Pharmacol* 1999; **50**: 827-31.

25. **Figura** N, Di Cairano G, Lore F, Guarino E, Gragnoli A, Cataldo D, Giannace R, Vaira D, Bianciardi L, Kristodhullu S, Lenzi C, Torrecelli V, Orlandini G, Gennari C. The infection by *Helicobacter pylori* strains expressing CagA is highly prevalent in women with autoimmune thyroid disorders. *J Physiol Pharmacol* 1999; **50**: 817-826.

26. **Fucuda** Y, Bamba H, Okui M et al. *Helicobacter pylori* infection increases mucosal permeability of the stomach and intestine. *Digestion.* 2001; **63** (Suppl): 93-6.

27. **Füreder** W, Agis H, Willheim M, Banki HC, Maier U, Kishi K, Müller MR, Czerwenka T, Radaszkiewicz T et al. Differential expression of complement receptors on human basophils and mast cells. Evidence for mast cell heterogenity and CD88/C5aR expression on skin mast cells. *J Immunol* 1995, **155**: 3152-3160.

28. **Gaig** P, Garcia-Ortega P, Enrique E, Richart C. Successful treatment of chronic idiopathic urticaria associated with thyroid autoimmunity. *J Invest Allergol Clin Immunol* 2000; **10**: 342-345.

29. **Grattan**, CEH, Wallington, TB, Warin RP. A serological mediator in chronic idiopathic urticaria: a clinical and histological evaluation. *Br J Dermatol* 1986; **114**: 583-90

30. **Greaves** MW. Chronic urticaria. *J Allergy Clin Immunol* 2000; **105**: 664-672.

31. **Greaves** MW. Chronic idiopathic urticaria and *Helicobacter pylori* – not directly causative, but could there be a link? *ACI Int* 2001; **13**: 23-26.

32. **Greaves** MW. Pathophysiology of chronic urticaria. *Int Arch Allergy Immunol* 2002; **127**: 3-9

33. **Haupl** T, Landgraf S, Netusil P, Biller N, Capiau C, Desmons P, Hauser P, Burgmeister GR. Activation of monocytes by three OspA vaccine candidates: lipoprotein OspA is a potent stimulator of monokines. *FEMS Immunol Med Microbiol* 1997; **19**: 15-23.

34. **Hellgren** L. The prevalence of urticaria in the total population. *Acta Allergologica.* 1972; **27**: 236-40.

35. **Henz** BM, Zuberbier T. Causes of urticaria. In Henz BM, Zuberbier T, Grabbe J, Monroe E, eds. Urticaria: Clinical, Diagnostic and Therapeutic Aspects. Berlin: Springer-Verlag, 1998: 19-38.

36. **Hide**, M, Francis DM, Grattan, CEH, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause for histamine release in chronic urticaria. *N Eng J Med* 1993; **328**: 1599-604.

37. **Hídvégi** B, Gonzáles-Cabello R, Temesvári E, et al. The effect of heat-inactivated *Helicobacter pylori* on the blastogenic response of peripheral blood mononuclear cells of patients with chronic urticaria. *Int Arch Allergy Immunol.* 2001; **126**: 167-172.

38. **Hizal** M, Tuzun B, Wolf R, Tuzun Y. The relationship between *Helicobacter pylori* IgG antibody and autologous serum test in chronic urticaria. *Int J Dermatol* 2000; **39:** 443-445.

39. **Hunt** PJ, Marshall SE, Weetman AP, et al. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clin Endocrinol* 2001; **55**: 491-9.

40. **Husz** S. Az urticaria patogenezise, klinikai formái és kezelése. *Medicus Anonymus* 2002; **5**: 37-41.

41. **Kalabay** L, Fekete B, Czirják L, Horváth L, Daha RM, Veres A, Fónay G, Horváth A, Viczián Á, Singh M, Hoffer I, Füst G, Romics L, Prohászka Z. *Helicobacter pylori* infection in connective tissue disorders is associated with high levels of antibodies to mycobacterial Hsp65 but not to human Hsp60. *Helicobacter* 2002; **7**: 250-257.

42. **Kalas** D, Prónai L, Ferenczi K, Pálos G, Daróczy J: A *Helicobacter pylori* fertőzés és a gastrointestinalis krónikus urticaria összefüggésének vizsgálata. *Orv Hetil* 1996;

137:1969-1972.

43. **Keenan** J, Oliaro J, Doigan N, Potter H, Aitken G, Allardyce R, Roake J. Immune response to an 18-kilodalton outer membrane antigen identifies lipoprotein 20 as a *Helicobacter pylori* vaccine candidate. *Infect Immun* 2000a; **68**: 3337-3343.

44. **Keenan** JI, Allardyce RA, Bagshaw PF. A role for the bacterial outer membrane in the pathogenesis of *H. pylori* infection. *FEMS Microbiol Lett* 2000b; **182**: 259-264.

45. **Kostrzynska** M, O'Toole PW, Taylor DE, Trust TJ. Molecular characterisation of a conserved 20-kilodalton membrane-associated lipoprotein antigen of *Helicobacter pylori. J Bacteriol* 1994;**176**:5938-5948.

46. **Lanigan** SW, Short P, Moult P. Association of chronic urticaria and thyroid autoimmunity. *Clin Exp Dermatol.* 1987; **12**: 335-338.

47. **Leznoff** A, Josse RG, Denburg J, Dolovich J. Association of chronic urticaria and angioedema with thyroid autoimmunity. *Arch Dermatol* 1983; **119**: 636-640.

48. **Leznoff** A, Sussman GL. Syndrome of idiopathic chronic urticaria and angioedema with thyroid autoimmunity: A study of 90 patients. *J Allergy Clin Immunol* 1989; **84**: 66-71.

49. **Liutu** M, Kalimo K, Uksila J, Kalimo H. Etiologic aspects of chronic urticaria. *Int J Dermatol* 1998; **37**: 515-519

50. **Lutton** DA, Bamford KB, O'Loughlin B, Ennis M. Modulatory action of Helicobacter pylori on histamine release from mast cells and basophils in vitro. *J Med Microbiol* 1995; **42**: 386-393

51. **Mai** UEH, Perez-Perez GI, Wahl LM, Wahl SM, Blaser MJ, Smith PD. Soluble surface proteins from *Helicobacter pylori* activate monocytes/macrophages by a

lipopolysaccharide-independent mechanism. *J Clin Invest* 1991; **87**: 894-900.

52. **Matysiak-Budnik** T, Heyman M. Food allergy and *Helicobacter pylori*. *J Pediatr Gastroenterol Nutr* 2002; **34**:5-12.

53. **Mollenkopf** C, Steininger H, Weineck G, Meyer M. Gastritis: Immunohistochemischer Nachweis der spezifishen and unspecifischen Imunabwehr gegen Helicobacter pylori. Z. Gastroenterol. 1990; **28**: 327-331.

54. **Neish** AS, Gewirtz AT, Zeng H et al. Prokaryotic regulation of epithelial responses by inhibition of IkappaBalpha ubiquitanation. *Science* 2000; **289**: 1560-3.

55. **Negrini** R, Savio A, Appelmelk BJ. Autoantibodies to gastric mucosa in *Helicobacter pylori* infection. *Helicobacter* 1997; **2(S1)**: 13-6.

56. **Niimi** N, Francis DM, Kermani F, et al. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol.* 1996; **106**: 1001-1006.

57. **O'Donnell** BF, O'Neill CM, Francis DM, et al. Human leucocyte antigen class II associations in chronic idiopathic urticaria. *Br J Dermatol* 1999; **140:** 853-8.

58. **Realdi** G, Dore MP, Fastame L. Extradigestive manifestations of *Helicobacter pylori* infection. Fact and fiction. *Dig Dis Sci* 1999; **44**: 229-36.

59. **Rebora**, A, Drago F, Parodi A: May *Helicobacter plyori* be important for dermatologist? *Dermatol.* 1995; **191**:6-8.

60. **Reinauer** S, Megahed M, Goerz G, Ruzicka T, Borchard F, Suasanto F, Reinauer H. Schönlein-Henoch purpura associated with gastric Helicobacter pylori infection. *J Am Acad Dermatol* 1995; **33**: 876-879.

61. **Ryhal** B, DeMera R, Shoenfeld Y, Peter JB. Gersshwin ME. Are autoantibodies present in patients with subacute and chronic urticaria? *J Invest Allergol Clin Immunol* 2001; **11**: 16-20.

62. **Sabroe** RA, Francis DM, Barr RM, Black AK, Greaves MW. Anti-FcεRI autoantibodies and basophil histamine releasability in chronic idiopathic urticaria. J *Allegy Clin Immunol* 1998; **102**: 651-658.

63. **Sabroe** RA, Grattan CEH, Francis DM, et al. The autologous serum skin test: a screening test for autoantibodies in chronic urticaria. *Br J Dermatol* 1999a; **140**: 446-52.

64. **Sabroe** RA, Poon E, Ochard GE, Lane D, Francis DM, Barr RM, et al. Cutaneous inflammatory cell infiltrate in chronic idiopathic urticaria: comparison of patients with and without anti- FcεRI or anti-IgE autoantibodies. *J Allegy Clin Immunol.* 1999b; **103**: 484-493.

65. **Schnyder** B, Helbling A, Pichler WJ. Chronic idiopathic urticaria: natural course and association with *Helicobacter pylori* infection. *Int Arch Allergy Immunol* 1999; **119:** 60-3.

66. **Tamássy** K, Simon L, Mégraud F. *Helicobacter pylori* infekció magyarországi epidemiológiája (szeroepidemiológiai összehasonlitó tanulmány). *Orv. Hetil.* 1995; **136**: 1387-91.

67. **Tebbe** B, Geilen CC, Schulzke J-D, Bojarski C, Radenhausen M, Orfanos CE. *Helicobacter pylori* infection and chronic urticaria. *J Am. Acad. Dermatol.* 1996; **34**: 685-686

68. **Trachsel** C, Pichler WJ, Helbling A. Stellenwert von Laboruntersuchungen und Triggerfactoren bei der chronischen Urtikaria. *Schweiz Med Wochenschr* 1999; **129**:

1271-79.

69. **Turktas** I, Gokcora N, Demirsoy S. et al. The association of chronic urticaria and angioedema with autoimmune thyroiditis. *Int J Dermatol* 1997; **36:** 187-190.

70. **Vaarala** O, Alfthan G, Jauhiainen M, Leirisalo-Repo M, Aho K, Palouso T. Crossreaction between antibodies to oxidized low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet* 1993; **341**: 923-925.

71. **Valle** J, Seppälä k, Sipponen P, Kosunen T: Disappearance of gastritis after eradication of *Helicobacter pylori. Scand J Gastroenterol* 1991; **26**: 1057-1065.

72. **Valsecchi** R, Pigatto P. Chronic urticaria and *Helicobacter pylori. Acta Derm Venereol* 1998; **78**: 440-2.

73. **Wedi** B, Wagner S, Werfel T, Manns MP, Kapp A. Prevalence of *Helicobacter pylori*-associated gastritis in chronic urticaria. *Int Arch. Allergy Immunol* 1998; **116**: 288-294.

74. **Wustlich** S, Brecler R, Luger TA, et al. *Helicobacter* as a possible bacterial focus of chronic urticaria. *Dermatology* 1999; **198**: 130-2.

Publications supporting the thesis

1. **Bakos** N, Mészáros Cs, Debreczeni M: A chronicus urticaria vizsgálata. (Investigation of chonic urticaria) *Orvosi Hetilap* 1990; **131**: 1183-1186.

2. **Bakos** N, Mészáros Cs, Hunyadi J: Immunpathológiai vizsgálatok chronicus urticariaban.(Immunopathological studies in chronic urticaria.) *Orvosi Hetilap* 1995; **136**: 2603–2608.

3. **Bakos** N, Szántó H: A Helicobacter pylori patogenetikai szerepe krónikus urtikáriában. (Pathogenetic role of *Helicobacter pylori* in chronic urticaria.) *Bőrgyógy. és Venerol. Szemle* 1998; **74**: 9-13.

4. **Bakos** N, Hillander M: Vizsgálatok autoimmun urtikáriában. (Investigations on autoimmune urticaria.) *Bőrgyógy. és Venerol. Szemle* 2002; **78**: 157-160

5. **Bakos** N, Hillander M: Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria. *Int. J. Dermatol.* (in press)

6. **Bakos** N, Fekete B., Prohászka Z, Füst G, Kalabay L: High prevalence of IgG and IgA antibodies to 19 kd *Helicobacter pylori* associated lipoprotein in chronic urticaria. *Allergy* (in press)

Abstracts in Indexed Journals

1. **Bakos** N, Mészáros Cs. Investigations in chronic urticaria. *Allergy* 1996; S41: 236.

2. **Bakos** N, Hillander M, Viczián Á, Fekete B, Füst G. Investigation of chronic urticaria associated with *Helicobacter pylori*. *Immunol Lett* 2000; **73**: 276.

3. **Bakos** N, Szemere P. The role of *Helicobacter pylori* in pathogenesis of chronic urticaria. *Allergy* 2002; **57**: S73: 314.

Pubications indirectly connected with the thesis

1. **Bakos** N, Remenyik É, Debreczeni M, Mészáros Cs: Alimentáris urticaria vizsgálata. (Study of alimentary urticaria.) *Bőrgyógy. és Venerol. Szemle* 1989; **65**: 49-53.

2. **Bakos** N, Mészáros Cs, Debreczeni M: A basophil degranuláció vizsgálata Penicillin allergiás betegekben. (Study of basophil degranulation in penicillin-allergic patients.) *Bőrgyógy. és Venerol. Szemle* 1990; **66**: 201-204.

3. **Bakos** N: Második generációs, nonszedativ antihisztaminok. (Second-generation, non-sedative antihistamines) *Orvosi Hetilap* 1994; **135**: 16-20.

4. **Bakos** N, Mészáros Cs, Hunyadi J: Antihisztaminok alkalmazása a bőrgyógyászatban. (Application of antihistamines in dermatology) *Gyógyszereink* 1994; 44; 1-5.

5. **Bakos** N, Mészáros Cs, Hunyadi J: Perubalzsam érzékenység elõfordulása ulcus cruris venosum esetén.. (Occurence of Peru balsam sensitivity in ulcus cruris venosum.) *Bőrgyógy. és Venerol. Szemle* 1995; **7:** 25-30.

6. **Bakos** N: Hidegagglutininémiához társuló szekunder–hideg urtikária gyermekkori esete.. (A case of secondary cold urticaria associated with cold agglutinaemia in childhood.) *Bőrgyógy. és Venerol. Szemle* 1996; **72**: 25-28.

7. **Bakos** N, Szemere P: A mastocyták szerepe az I. típusú allergiás reakció és gyulladás kialakulásában. (The role of mast cells in the development of an allergic reaction of type I and inflammation.) *Magy Belorv Arch* 1996; **49**: 199–206.

8. **Bakos** N, Szemere P: Immunpathológiai eltérések atopias dermatitisben. (Immunopathological deviations in atopic dermatitis.) *Orvosi Hetilap* 1997; **138**: 587–592

9. **Bakos** N, Szemere P: Az atopias dermatitis terápiája. (The therapy of atopic dermatitis.) *Orvosi Hetilap.* 1997, **138**: 1121-1127

10. **Bakos** N, Mészáros Cs, Hunyadi J: Prognosztikai faktorok vizsgálata atopias dermatitisben. (Study of prognostic factors in atopic dermatitis.) *Bőrgyógy. és Venerol. Szemle* 1997; **73**: 119-124.

11. **Bakos** N: Pollenszenzibilizáció szerepe a bõrgyógyászati allergiákban. (The role of pollen sensitization in dermatological allergies.) *Allergológia és Klinikai Immunológia* 1999; **2:** 68-123.

12. **Bakos** N: A bőrgyógyászati allergiák modern terápiája. (The modern therapy of dermatological allergies.) *Gyógyszereink.* 2000; **50**: 97-100.

13. **Bakos** N: Zöldség és gyümölcsallergia. (Vegetable and fruit allergies.) *In: Magyar Táplálékallergia és Táplálékintolerancia Adatbank.* 127-133. Zoo-Vita, Budapest. 2000

14. **Bakos N.** Nékám K: Növényi és állati eredetű táplálékok közötti allergiás keresztreakciók. (Allergic cross-eactivity between foodstoffs of plant and animal origin.) *Orvosi Hetilap* 2000; **141**: 657-662.

15. **Bakos** N, Szemere P, Cserni I: Az atopias dermatitis terápiája. (The therapy of atopic dermatitis.) *Útmutató.* 2002; **6**: 321-325.

16. **Bakos** N, Minker E, Cserni I: Krónikus urticaria.(Chronic urticaria.) *Útmutató.* 2002; **6**: 334-335.

17. **Bakos** N. A krónikus urtikária pathogenezise és klinikai formái. (Pathogenesis and clinical forms of chronic urticaria.) *Medicus Anonymus* 2003; (in press)

Paper and Poster Presentations

1. **Bakos** N, Mészáros Cs. Investigations in chronic urticaria. XV Congress of the European Academy of Allergology and Clinical Immunology (Madrid, Spain, 1996, Poster)

2. **Bakos** N, Hillander M, Viczián Á, Fekete B, Füst G. Investigation of chronic urticaria with *Helicobacter pylori.* 14th European Immunology Meeting EFIS (Poznan, Poland, 2000, Poster)

3. **Bakos** N. Gasztrointesztinális kórképek lehetséges bőrgyógyászati vonatkozásai a „Helicobacter korában". (Dermatologic disorders connecting with gastrointestinal aleteration in "Helicobacter period") Meeting of Hospital Istvan (Budapest, 2001, Paper)

4. **Bakos** N. *Helicobacter pylori* és táplálék allergia. (*Helicobacter pylori* and food allergy) Medipharma 2002 (Budapest, 2002, Paper)

5. **Bakos** N, Fekete B, Prohászka Z, Füst G, Kalabay L. A 19 kD *Helicobacter pylori*-asszociált lipoprotein ellenes IgG és IgA antitestek fokozott prevalenciája krónikus urtikáriában. (High prevalence of *Helicobacter pylori* associated IgG and IgA lipoprotein in chronic urticaria.) Congress of Hungarian Society of Dermatology (Debrecen 2002, Paper)

6. **Bakos N.** The role of *Helicobacter pylori* in pathogenesis of chronic urticaria. XXXI Congress of the European Academy of Allergology and Clinical Immunology (Naples, Italy, 2002, Poster)

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ANNEX

(Full papers)